INFLUENCE OF SALIVARY PH AND UREA LEVEL ON CALCULUS FORMATION: A CLINICO-BIOCHEMICAL STUDY

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ABSTRACT

Background: Estimating the levels of salivary pH and urea aids in determining their role in rate of calculus formation. This study is aimed at evaluating the pH and urea level of saliva, determine its role as a risk factor for periodontal disease and thus evaluate its suitability as a diagnostic marker of periodontal disease.

Materials & methods:
Based on objective evaluation of the oral hygiene condition, subjects were divided into three groups:

Group A- mild / no calculus levels (control group, having calculus index score of 0.0 to 0.6)

Group B- moderate calculus levels (having calculus index score of 0.7 to 1.8)

Group C- heavy calculus levels (having calculus index score of 1.9 to 3.0)

Pre-operatively pH and urea levels were recorded of un-stimulated whole saliva. Calculus and plaque scores were recorded amongst the groups, and thorough oral prophylaxis was performed in all the groups.

Results: Salivary pH and urea levels have an influence on calculus formation.

Introduction
Plaque and calculus have for long been at the forefront of the etiology of psseriodontal diseases. While plaque is the primary causative factor in the initiation of periodontal destruction, calculus has a secondary role in this mechanism and acts as a retentive surface for more plaque accumulation, thereby causing more destruction.

Calculus is a significant pathogenic factor in periodontal disease for a number of reasons:

1. It acts as the retention site of plaque and brings plaque bacteria closer to the supporting tissues. 2. It provides a fixed nidus for continuous accumulation of plaque and calculus.
3. It interferes with the local self-cleansing mechanism.
4. It makes plaque removal more difficult for the individual.
5. It acts as a reservoir for irritating substances like endotoxin and products of tissue lysis because of its permeability and porous nature.
6. Calculus extends the bacterial front and shifts the bacterial challenge and the zone of destruction more apically.

The saliva is a complex fluid containing a variety of mucosal host defense factors from the different salivary glands and the crevicular fluid.[1] The pH of saliva has a wide range of 5-8. Studies had reported the importance of alkaline pH for deposition of calcium phosphate, thereby promoting plaque mineralization.[2]

Saliva is a biological environment, important for the physiology of the mouth. It achieves its mechanical functions of cleaning and protection through various physical and biochemical mechanisms. For keeping the electrochemical reaction in oral homeostasis, except the bicarbonate, phosphate and protein buffer, other compounds or enzymes participate, having a buffer role. This group includes urea, salivary amylases and fluorides as prophylactic buffers.

The importance of salivary urea was acknowledged early in dental literature. [3,4] The pH-raising effect of intraoral urea application was first described by Stefan.[5] This author found that in both in vivo and in vitro urea could raise plaque pH up to pH 9 and that the addition of 40–50% urea to carbonhydrates largely overcame the pH-lowering effect for up to 24 h.

The normal concentrations of urea in unstimulated and stimulated whole saliva are 3.3 and 2.2 mmol/l, respectively.[6] Urea has a dual effect: it inhibits the metabolism and multiplication of bacteria in the saliva on the one hand, and on the other hand it indirectly affects neutralizing the acids in the oral environment, thus participating in maintaining the salivary acidobasic balance, which it actually owes to its buffer capacity.7,8

Urea, which is present in blood and saliva, is an organic substance synthesized from amino acids and carbon dioxide. Some oral microbes hydrolyze salivary and dietary urea via the enzyme urease to produce ammonia and carbon dioxide, which results in an increase in plaque pH.[9,10]

The ureolytic pH response (an increase in plaque pH by the production of ammonia from urea) promotes calculus formation by increasing the saturation degree of calcium phosphate in plaque fluid.[11]

Thus, there is general agreement that supragingival deposits derive most of their mineral content and part of their matrix from saliva. It would, therefore, seem logical to examine salivary composition in heavy and light calculus formers as a means of identifying factors responsible for individual susceptibility. The present study was, therefore, designed to evaluate the effect of salivary pH and urea level on calculus formation in heavy as well as mild calculus formers.

MATERIALS AND METHODS
The subjects constituted the volunteers taken from among the patients coming to the out-patient unit of Department of Periodontics, Terna Dental College, Navi-Mumbai.

The inclusion criteria followed were:
1. Age group 18-55 years
2. Systemically healthy
3. Not undergoing any drug therapy
4. Not had any periodontal therapy in the past 3 months, and no discernible calculus formation for at least a year
5. Not had any periodontal therapy in the past 3 months, but forming calculus at a rate requiring frequent oral prophylaxis over a year
6. Willingness to participate in the study

Based on objective evaluation of the oral hygiene condition and the Calculus Index (subpart of OHI-S index), subjects were divided into three groups:
Group A- Mild calculus formers (mild / no calculus levels (control group, having calculus index score of 0.0 to 0.6)

Group B- Moderate calculus levels (having calculus index score of 0.7 to 1.8)

Group C- Heavy calculus levels (having calculus index score of 1.9 to 3.0)
10 subjects were assigned to each group. Informed consent was obtained from each subject. The subject refrained from oral hygiene, eating, drinking, or smoking for at least 2 hours before clinical parameters were recorded.

Saliva Sampling
The subject was asked to sit still and relaxed, with head slightly tilted down, and minimal oral musculature activity, including swallowing. Unstimulated whole saliva was allowed to pool in the floor of the mouth over 5 minutes and was asked to expectorate in a sterile container as passively as possible.

Salivary pH was recorded by using pH indicator strips [Dental Saliva pH indicator strips pH 6.5 - 9.0; gradation 0.5; color coded] **(figure 1)** as soon as saliva was collected in a sterile container.

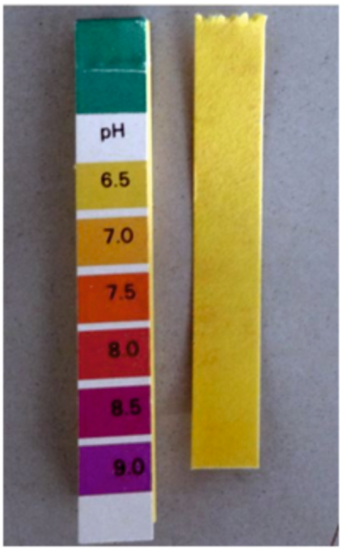

Figure 1: Salivary pH indicator strips

Thereafter, biochemical estimation of salivary urea was performed with the help of manual modified Berthelot method using Urea Kit* [Reckon, (Made in India)]. **(figure 2)**

Figure 2: Salivary urea kit

The calculus scores were recorded according to Calculus index (sub part of Oral Hygiene Simplex- Index). Thereafter, plaque was disclosed (using Alphaplac® DPIIndia) and plaque scores recorded based on Löe and Silness Plaque index.

STATISTICALANALYSIS:
The information was tabulated and Inter-group Comparison (Anova Test) and Multiple Comparison (Bonferroni Test) was performed for pH and urea levels with the help of SPSS package (SPSS, Inc, Chicago, IL, USA) version 17.0.

RESULTS
At the end of the study, of 30 subjects, the mean and standard deviation for Group A, Group B and Group C are shown in table 1 and the Bonferroni test was shown in table 2, there was no statistically significant difference between pH levels in the groups between Group A and Group B (p=0.317) but highly significant between Group A and Group C (p= 0.000).

When urea levels were compared it was found there was statistically significant difference between Group A and Group B (p= 0.002) also between Group A and Group C (p= 0.000).

Table-1: Mean values of salivary Urea, pH, PI (Plaque Index) and GI (Gingival Index) in Group A, Group B and Group C.

		N	Mean	Std. Deviation	Std. Error
Urea	low cal	10	21.6000	2.79682	.88443
	mod cal	10	29.8000	5.76965	1.82452
	high cal	10	35.9000	5.48635	1.73494
	Total	30	29.1000	7.59015	1.38577
pH	low cal	10	7.1700	.43982	.13908
	mod cal	10	7.4600	.31340	.09911
	high cal	10	7.9500	.39791	.12583
	Total	30	7.5267	.49684	.09071
Pi	low cal	10	.8050	.15241	.04820
	mod cal	10	1.2130	.30419	.09619
	high cal	10	1.7000	.46538	.14717
	Total	30	1.2393	.49153	.08974
Gi	low cal	10	.1700	.09475	.02996
	mod cal	10	.7690	.36026	.11392
	high cal	10	1.6400	.59554	.18833
	Total	30	.8597	.72798	.13291

Multiple Comparisons
Bonferroni

Table-2: Comparison of salivary urea, pH, PI (Plaque Index) and GI (Gingival Index) among group A, Group B and Group C

Dependent Variable	(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.
Urea	low cal	mod cal	-8.20000*	2.17885	.002
		high cal	-14.30000*	2.17885	.000
	mod cal	low cal	8.20000*	2.17885	.002
		high cal	-6.10000*	2.17885	.028
	high cal	low cal	14.30000*	2.17885	.000
		mod cal	6.10000*	2.17885	.028
pH	low cal	mod cal	-.29000	.17321	.317
		high cal	-.78000*	.17321	.000
	mod cal	low cal	.29000	.17321	.317
		high cal	-.49000*	.17321	.026
	high cal	low cal	.78000*	.17321	.000
		mod cal	.49000*	.17321	.026
Pi	low cal	mod cal	-.40800*	.14885	.032
		high cal	-.89500*	.14885	.000
	mod cal	low cal	.40800*	.14885	.032
		high cal	-.48700*	.14885	.009
	high cal	low cal	.89500*	.14885	.000
		mod cal	.48700*	.14885	.009
Gi	low cal	mod cal	-.59900*	.18137	.008
		high cal	-1.47000*	.18137	.000
	mod cal	low cal	.59900*	.18137	.008
		high cal	-.87100*	.18137	.000
	high cal	low cal	1.47000*	.18137	.000
		mod cal	.87100*	.18137	.000

DISCUSSION
Considerable effort has been put in by various authors in the past to understand the mechanism of calculus formation, and the factors influencing it.

The present study was carried out to evaluate the influence of salivary pH and

urea levels on calculus formation in heavy as well as mild calculus formers. It was evident that there was a significant difference in the salivary urea concentrations of all the groups.

However, only 60% of the subjects with heavy calculus reported with high urea concentration (≥ 36 mg/dL), while the remaining subjects had normal or only marginally high urea levels. It was found that there is a significant correlation between urea concentration in saliva and corresponding pH values in heavy calculus formers, which reflected in considerable amount of calculus deposition in these subjects.

The importance of alkaline pH for deposition of calcium phosphate, thereby promoting plaque mineralization was also reported by Wong L et al.[2] The salivary pH in chronic generalized periodontitis was found to be statistically significantly high compared to healthy gingiva.1 The present study demonstrated a positive and significant correlation between urea levels and calculus formation in both the groups, which is in accordance with the study conducted by Gupta et al.[12] and Ramisetti et al.[13]

Most probably the nitrogenous material in saliva is broken down to smaller units such as the conversion of urea to ammonia, amino acids to ammonia and other products, and even the breakdown of mucin and other proteins to smaller units.2 While oral ureolytic activity is ubiquitous,[14] it varies quantitatively between individuals' plaque. This variation is probably related to variations in the flora, since common plaque organisms differ greatly in their capacity to degrade urea. Plaque may be regarded as a promotion of a natural process.

Focal alkalinization has long been considered a factor in the natural mineralization of plaque during its transformation to calculus, with the production of ammonia from salivary urea one of the means by which the pH is raised.[15] Application of urea solutions to plaque in situ causes a rapid rise in supragingival plaque pH. This rise has been attributed to the formation of ammonia by the ureolytic activity of the plaque bacteria. Both the levels of urea in saliva and of ammonia in plaque are sufficient to account for the high pH of fasting plaque.[16]

In contrast to our study, a double-blind, cross over study of three months' frequent use of sugar free chewing gum-with or without urea-neither promotes nor inhibits calculus formation.[17]

However, the variation in calculus formation in different individuals may also be related to variation in salivary flow rate in different regions of the oral cavity. Other components of saliva may also contribute to calculus deposition, such as Ca2+ and P3- super saturation, buffering capacity, protein content, and certain organic acids.[12]

The diagnosis of active phases of periodontal disease and the identification of patients at risk for active disease represents a challenge for both clinical investigators and clinicians. Saliva, like blood, contains an abundance of protein and nucleic acid molecules that reflects physiological status; however, unlike other bodily fluids, salivary diagnostics offer an easy, inexpensive, safe, and noninvasive approach for disease detection, and possess a high potential to revolutionize the next generation of diagnostics.[18]

CONCLUSION:

It can be concluded that salivary urea has a major influence on calculus formation, and even though evidence shows that pH has a role in calculus formation, the present study did not show significant relation between pH and calculus formation, which could be because of the small sample size. Therefore further investigation using a large sample size may reveal the significance of pH in calculus formation.

Also, though we have long way to go, the use of saliva-based oral fluid diagnostics shows a promising future in diagnostic & prognostic field in Periodontology.

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